wherein said encapsulation means comprises a coacervate, and wherein release of said encapsulated [expression] <u>viral</u> vector from said encapsulation means transfects a cell.

Please add the following new claim:

49. (New) The composition of claim 1, wherein the nucleic acid encodes a polypeptide which inhibits cell proliferation.

<u>REMARKS</u>

Claims 1, 2, and 4-49 are pending upon entry of the above amendment. Support for the amendments above may be found throughout the specification. No new matter is being added. Claim 3 has been cancelled without prejudice. Applicants reserve the right to prosecute claims of similar or differing scope to the originally presented claims in subsequent applications. Applicants will now address the issues raised in the Office Action in the order presented therein.

<u>Specification</u>

The abstract is objected to because of the inclusion of the term 'said bioactive substance'. Applicants have replaced the word 'said' with 'the', thereby obviating this objection.

<u>Claim Rejections under 35 U.S.C. 101</u>

Claim 39 is rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter. Applicants have amended this claim to recite steps of a method for preparing a pharmaceutical preparation. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

<u>Claim Rejections Under 35 U.S.C. §112, first paragraph</u>

Claims 17-20, 29, 30-34, 39, 47, and 48 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a coacervate microsphere for controlled release of a recombinant expression vector or a recombinant virus from said microsphere, and the

expression of a recombinant protein in transfected cells *in vitro* and in localized injected mouse tissues or implanted tumor cells, a gene delivery system, and a method of delivery utilizing the same coacervate microsphere, allegedly does not reasonably provide enablement for the intended therapeutic uses of the same. Applicants respectfully traverse this rejection.

The Examiner cites Dang et al. as stating that advancement in all fields including gene delivery, gene expression, immune manipulation, and the development of molecular targets is needed to make gene therapy a reality. It appears that the Examiner is requiring a demonstration of superior efficacy and safety sufficient to make the claimed methods suitable for clinical use in humans. However, the Examiner is reminded that, as set forth in MPEP 2107.02 (V), it is for the Patent Office to determine if Applicants have enabled one of skill in the art to make and use the invention, while safety and efficacy are issues to be resolved by the FDA.

Applicants have shown that coacervates incorporating a nucleic acid and a delivery agent, as set forth in the present claims, can transfect cells and result in expression of nucleic acids in an expression cassette. Vectors used in established therapies may be incorporated into coacervates for sustained delivery, allowing the fruits of prior studies to be used directly in the presently claimed compositions and methods. The Examiner has not provided any evidence in support of a position that the claimed coacervates would be ineffective if the expression cassette included, for example, a nucleic acid that has been therapeutically beneficial when administered by another vehicle.

By way of example, number of therapeutically beneficial nucleic acids and treatment regimens are described in the very references cited by the Examiner against the present claims. Dang et al. features "the pioneering work of Anderson and Blaese" on the first page of their article. In that experiment, the ADA gene was transduced in the lymphocytes of infants with ADA-deficient SCID. Four years later, those patients continued to produce lymphocytes containing ADA cDNA. Although the level of expression was not sufficient to *cure* the deficiency, and continued treatment with PEG-ADA was required, Dang et al. does not indicate that the long-term expression of ADA by the patient's lymphocytes was not sufficient to result in some improvement in the patient's condition relative to no treatment. Moreover, recent research described by Kawamura et al., *J. Immunol.* 1999, 2256-61, and Ariga et al., *Nippon Rinsho* 1997,

1572-9, abstracts of which are provided herewith, suggest that measurable improvement can indeed be observed in such patients. Applicants respectfully point out that the claimed methods need not provide a 100% effective, reliable, and complete cure of a condition in order to be a useful and enabled treatment for a condition. Thus, absent a specific teaching that the presently claimed compositions and methods would be ineffective in treating ADA-deficient SCID, one of skill in the art would expect that coacervates could be used to deliver the ADA gene and induce a therapeutically effective response, even if conjoint therapy would be required for clinically acceptable and complete control of the condition.

On page 471 of Dang et al., experiments by Hung et al. are summarized. Hung et al. found that cationic liposomes and adenoviral vectors can effectively deliver E1A to tumor cells in mice, resulting in suppression of tumor growth and longer survival of the mice. These results spurred a Phase I trial which has shown that the E1A gene can be expressed in treated patients. Although further trials will be required to evaluate therapeutic efficacy, these results suggest that such treatment is effective in treating cancer. No evidence has been cited to indicate that the currently claimed coacervates, modified according to the precepts of Hung et al., would not similarly demonstrate some level of efficacy.

Although Dang et al. narrowly define the characteristics of an 'ideal vector', decry sub-optimal vectors, and question the availability of long term, stable gene expression and of efficient gene delivery to target tissues, Dang et al. describes several approaches to gene therapy that have shown efficacy *in vivo*, clearly indicating that current methodology can be used for effective *in vivo* gene therapy. The Examiner is reminded that a therapy, to be patentable, need not outperform any existing therapy, nor singlehandedly cure a disease, but need only be useful as a part of a therapeutic regimen, or ameliorate one or more symptoms of a condition. Applicants believe that the presently claimed compositions and methods, taken with the knowledge of those of skill in the art and prior research using other gene delivery vehicles, would lead one of skill in the art to expect that coacervates incorporating a nucleic acid and a delivery agent could be successfully used in the treatment of disease.

The Examiner then cites Wivel and Wilson, who state that "[o]ne of the major challenges still confronting the field is the design of more efficient vectors. The gene delivery systems being

used today will undoubtedly be seen as crude when compared with future developments. It is unlikely that there will ever be a universal vector, but rather there will be multiple vectors specifically designed for certain organ sites and certain diseases.... It will be necessary to do much more fundamental research in cell biology, virology, immunology, and pathophysiology before vectors can be significantly improved." Even if true, these statements do not undercut the patentability of the present invention. Most, if not all, patent applications received by the U.S.P.T.O. describe technology which "will undoubtedly be seen as crude when compared with future developments", or "can be significantly improved" by further fundamental research. However, it is sufficient for patentability if the claimed compositions and methods are capable of inducing a desirable therapeutic effect. As stated above, a number of gene therapy techniques have shown efficacy, and a number of clinical trials are currently underway testing the safety and efficacy of gene therapy. Wivel and Wilson in no way suggest to one of skill in the art that the presently claimed compositions would be incapable of producing beneficial effects analogous to those that have been developed previously.

On page 488, Wivel and Wilson describe obtaining diminished disease in a mouse model of cystic fibrosis using an adenoviral vector. The fact that long-term gene expression is sub-optimal merely means that repeated doses may be necessary. Since many medical treatments, notably pharmaceuticals, require repeat administrations, this limitation does not represent an obstacle to patentability.

Eck and Wilson provide a considerable number of successful examples of therapeutic benefits derived from gene therapy. Table 5-1 lists a large number of approved clinical trials, many of which are supported by *in vivo* effectiveness in animal models. Additionally, on page 94, Eck and Wilson describe other successful *in vivo* uses of gene therapy. For example, *in vivo* gene transfer has been used to induce intimal hyperplasia in arterial walls, providing a model, though not a therapy, for disease. Nevertheless, this represents a useful and effective instance of gene transfer and expression *in vivo*. Eck and Wilson also describe the use of gene therapy with a vector encoding thymidine kinase with subsequent administration of ganciclovir for the successful inhibition of arterial hyperplasia. Table 5-2 on page 95 provides a number of gene-prodrug combinations useful for the treatment of cancer, some of which have been successful in

vivo in human and rodent models. In fact, one example of this type of therapy is currently in Phase III trials; the Phase I-II trials, summarized in the Klatzmann et al., Hum. Gene Ther. 1998, 2595-604 abstract provided herewith, demonstrated significant therapeutic benefits in the treatment of recurrent glioblastoma. None of the cited art would suggest to one of skill in the art that the presently claimed coacervates would be incapable of reproducing such effects.

Lastly, the Examiner cites Kalyanasundaram et al. as stating, with respect to the experiments described in the present application, that "these results did not demonstrate a superior gene transfer efficiency under these conditions." Again, Applicants point out that the enablement requirement does not require superiority, but merely effectiveness. The possibility of future modifications that might heighten the effectiveness of the claimed compositions and methods in no way suggests the present invention lacks enablement.

In summary, Applicants have claimed coacervates incorporating a nucleic acid and a delivery agent, and have shown that, when the delivery agent is an adenoviral vector, the nucleic acid can be released in a sustained manner, and transfected into and expressed by target cells in vivo. At the time of filing, a number of protocols were known, many of which are described in the references cited by the Examiner, which have demonstrated effective viral or liposome-mediated gene therapy for the treatment of a wide range of diseases, including cancer, cystic fibrosis, immune deficiency, and restenosis. The Examiner has cited no art which would suggest that these same protocols, if subjected to sustained release using the presently claimed coacervates, would not provide therapeutic benefits similar to those observed through traditional administration. Because such a wide range of delivery agents, such as liposomes and viruses, have been successfully used, and such a wide range of unrelated diseases have been successfully treated, if not cured, by gene therapy using a wide range of techniques for administration, Applicants submit that the pending claims are enabled throughout their scope. Accordingly, reconsideration and withdrawal of this rejection is respectfully requested.

Claim Rejections Under 35 U.S.C. §112, second paragraph

Claims 29, 32, 37, 40, and 42 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject

matter that Applicants regard as the invention. Applicants traverse this rejection to the extent it is maintained over the claims as amended.

Claim 29 has been amended to correct the lack of antecedent basis. Claim 32 has been amended to include the steps suggested by the Examiner. Claim 37 has been amended in accordance with the description of the engineered natural virus described at the top of page 4 of the specification to clarify the subject matter being claimed. Step (c) of claim 40 has been amended to clarify that the first and second solutions recited in step (a) have been altered according to step (b) before being combined in step (c). Claim 39 has been amended to recite a method including positive steps. Accordingly, Applicants submit that these claims, as amended, comply with 35 U.S.C. 112, second paragraph, and respectfully request withdrawal of this rejection.

Claim rejections under 35 U.S.C. 102

Claims 1-15, 17-21, 23-31, 33-34, 36-39, and 40-48 are rejected under 35 U.S.C. 102(a) as being anticipated by Kalyanasundaram et al. Applicants submit herewith a declaration under 37 C.F.R. 132 indicating that this reference is the work of the inventors and therefore is not prior art against the present application. Accordingly, withdrawal of this rejection is respectfully requested.

Claims 1, 2, and 48 are rejected under 35 U.S.C. 102(a) as being anticipated by Leong et al. or are rejected under 35 U.S.C. 102(e) as being anticipated by Roy et al. (U.S. Patent No. 5,972,707). Applicants respectfully traverse this rejection.

Claim 1 is directed to a composition for controlled release of a nucleic acid, including a coacervate; a nucleic acid incorporated in said coacervate; and a delivery agent incorporated in said coacervate, wherein the coacervate comprises a cationic molecule and an anionic molecule other than said nucleic acid. Claim 48 recites a gene delivery system for transfecting a cell with a viral vector, including encapsulation means for encapsulating a viral vector; delivery means for facilitating intracellular delivery of said encapsulated viral vector; wherein said encapsulation

means comprises a coacervate, and wherein release of said encapsulated viral vector from said encapsulation means transfects a cell.

Roy et al. and Leong et al. each teach coacervates containing nucleic acids, but do not apparently teach that the coacervates include an anionic molecule other than the nucleic acid, as recited by claim 1. Additionally, Roy et al. and Leong et al. do not apparently teach that the gene delivery system comprises a viral vector, as required by claim 48. Accordingly, Applicants submit that Roy et al. and Leong et al. fail to teach all the limitations of claims 1 and 48, and thus do not anticipate these claims. Reconsideration is respectfully requested.

Claim 29 is rejected under 35 U.S.C. 102(b) as allegedly being anticipated by Beer et al. Applicants respectfully traverse this rejection to the extent it is maintained over the claims as amended.

Claim 29 is directed to a gene delivery system for transducing cells of a host, comprising: a coacervate microsphere encapsulating at least a nucleic acid and a delivery agent for facilitating intracellular delivery of said nucleic acid, wherein upon administration of said coacervate microsphere to a host, controlled release of said nucleic acid results in transduction of cells of said host by said nucleic acid.

Beer et al. teach poly(lactic-glycolic)acid microspheres containing a recombinant adenovirus, but do not apparently teach coacervate microspheres as recited by amended claim 29. Thus, Beer et al. do not teach all the elements of claim 29, and cannot anticipate this claim. Accordingly, reconsideration is respectfully requested.

<u>Claim rejections under 35 U.S.C. 103</u>

Claims 1-28, 35, 36, 37, and 39 are rejected under 35 U.S.C. 103(a) as being unpatentable over Leong et al. or Roy et al. in view of Beer et al., Narayani et al., and Hedley et al. Applicants respectfully traverse this rejection to the extent it is maintained over the claims as amended.

The subject matter of claim 1 is described above. Claim 35 recites a kit containing a gene delivery system, including microspheres and instructions for using said microspheres, wherein said microspheres are comprised of a cationic molecule and an anionic molecule and said

microspheres encapsulate a virus. Claim 36 sets forth a coacervate microsphere for sustained release of a virus, including a coacervate of gelatin and alginate having a virus incorporated therein. Claim 39 is directed to a method for preparing a pharmaceutical preparation, comprising combining a pharmaceutically acceptable excipient with a coacervate of cationic and anionic molecules, wherein a recombinant virus is encapsulated in said coacervate.

Roy et al. and Leong et al. each teach coacervates containing nucleic acids, but do not apparently teach that the coacervates include an anionic molecule other than the nucleic acid, as recited by claim 1. Nor do Roy et al. and Leong et al. teach the incorporation of an anionic molecule, such as alginate, or a virus in the nanospheres.

In brief, Beer et al. teach poly(lactic-glycolic)acid microspheres containing a recombinant adenovirus. Narayani et al. teach alginate-coated gelatin capsules that survive the stomach and disintegrate in the intestine for oral delivery of protein and peptide drugs. Hedley et al. teach a preparation comprising microparticles having a polymeric matrix and a nucleic acid expression vector.

The Examiner suggests, first, that it would have been obvious to incorporate a virus in the coacervate nanospheres taught by Leong et al. or Roy et al., because AAV is more efficient at delivering a gene than a naked plasmid. However, the Examiner has provided no teachings which would suggest 1) that coacervate nanospheres can be formed around viral particles; and 2) that viral particles incorporated in coacervate nanospheres would retain their activity and facilitate intracellular delivery of nucleic acids.

Beer et al. prepare PLGA microspheres containing recombinant adenoviruses using a double emulsion technique, and note that the adenovirus is relatively large (>100 nm). Although Beer et al. suggest sustained release of adenovirus from a biodegradable polymer, they point out that they used gentle methods for encapsulation in consideration of mechanical forces on encapsulation (page 63 column 2), obtained spheres >10-20 μm in size, and never specifically suggest the use of coacervates. In contrast, the nanospheres taught by Leong et al. or Roy et al. are orders of magnitude smaller (200-750 nm), possibly too small to encapsulate large viral particles efficiently.

Moreover, Leong et al. espouse the use of non-viral delivery techniques for ease of synthesis, cell-tissue targeting, low immune response, and unrestricted plasmid size, and thus teach ***against*** this combination. Roy et al. and Leong et al. additionally rely on the interaction between negatively charged DNA and the positively charged cations in order to achieve coacervation (see, for example, Roy et al., column 6, lines 1-5). One of ordinary skill in the art would not expect this interaction to occur when the nucleic acid is inside a viral particle. For these reasons, this proposed combination lacks motivation or expectation of success.

The Examiner further suggests that one of skill in the art would be motivated to further protect the DNA in the composition by incorporating alginate with calcium as a crosslinking agent, following the teachings of Narayani et al. However, the Examiner fails to provide any motivation for combining the teachings of a reference relating to a coating for a capsule for the oral delivery of peptides and proteins with references describing microspheres and nanospheres for the delivery of nucleic acids and viruses. Neither Leong et al., nor Roy et al., nor Beer et al., nor even Narayani et al. teach or suggest oral administration of nucleic acids or vectors, no less administering such compositions as macroscale capsules. Moreover, the Examiner suggests this technique to avoid decomposition of the nucleic acids by nucleases. However, the techniques of Narayani et al. are useful for transporting a substance through the stomach and into the small intestine. While this may be effective for proteins and nucleic acids, the nucleases which decompose nucleic acids are primarily secreted by the pancreas into the small intestine and nucleosidases secreted by the small intestine itself. Thus, one of ordinary skill in the art, based on the cited teachings, would not recognize that this technique could be successfully applied to nucleic acids, even if it is effective for proteins and peptides. Accordingly, there is neither motivation to make this combination, nor a reasonable expectation of success, based on the art of record.

Lastly, the Examiner cites Hedley et al. as teaching microparticles comprising a polymeric matrix and DNA encoding an antigenic polypeptide. Although Hedley et al. suggest oral administration of nucleic acids, Hedley et al. overcome none of the above deficiencies of the cited art, and therefore cannot render the claimed subject matter obvious over the art of record.

For the above reasons, Applicants submit that the Examiner has failed to set forth a *prima facie* case of obviousness, and accordingly request reconsideration and withdrawal of this rejection.

Claims 30, 31, and 33 are rejected under 35 U.S.C. 103(a) as being unpatentable over Leong et al. or Roy et al. in view of Beer et al. and Narayani & Rao. Applicants respectfully traverse this rejection.

Claim 30 is directed to a method for delivering a nucleic acid to a host by administering to a host a composition comprising a coacervate, wherein the coacervate incorporates a nucleic acid contained in a transfer vector having at least one regulatory element, the coacervate comprises a cationic molecule and an anionic molecule other than the nucleic acid, the coacervate is a microsphere, and the coacervate incorporates a delivery agent, wherein administration of the composition results in controlled release of the transfer vector in vivo. Claims 31 and 33 are dependent thereon. The teachings of Beer et al., Roy et al., Leong et al., and Narayani & Roy are described above.

As noted above, the Examiner has provided no teachings which would suggest 1) that coacervate nanospheres can be formed around viral particles; and 2) that viral particles incorporated in coacervate nanospheres would retain their activity and facilitate intracellular delivery of nucleic acids. Although Beer et al. suggest sustained release of adenovirus from a biodegradable polymer, they point out that they used gentle methods for encapsulation in consideration of mechanical forces on encapsulation (page 63 column 2), obtained spheres >10-20 μm in size, and never specifically suggest the use of coacervates. In contrast, the nanospheres taught by Leong et al. or Roy et al. are orders of magnitude smaller (200-750 nm), possibly too small to encapsulate large viral particles efficiently.

Moreover, Leong et al. espouse the use of non-viral delivery techniques for ease of synthesis, cell-tissue targeting, low immune response, and unrestricted plasmid size, and thus teach ***against*** this combination. Roy et al. and Leong et al. additionally rely on the interaction between negatively charged DNA and the positively charged cations in order to achieve coacervation (see, for example, Roy et al., column 6, lines 1-5. One of ordinary skill in the art

would not expect this interaction to occur when the nucleic acid is inside a viral particle. For these reasons, this proposed combination lacks motivation or expectation of success.

The Examiner further suggests that one of skill in the art would be motivated to further protect the DNA in the composition by incorporating alginate with calcium as a crosslinking agent, following the teachings of Narayani et al. However, the Examiner fails to provide any motivation for combining the teachings of a reference relating to a coating for a capsule for the oral delivery of peptides and proteins with references describing microspheres and nanospheres for the delivery of nucleic acids and viruses. Neither Leong et al., nor Roy et al., nor Beer et al., nor even Narayani et al. teach or suggest oral administration of nucleic acids or vectors, no less administering such compositions as macroscale capsules. Moreover, the Examiner suggests this technique to avoid decomposition of the nucleic acids by nucleases. However, the techniques of Narayani et al. are useful for transporting a substance through the stomach and into the small intestine. While this may be effective for proteins and nucleic acids, the nucleases which decompose nucleic acids are primarily secreted by the pancreas into the small intestine and nucleosidases secreted by the small intestine itself. Thus, one of ordinary skill in the art, based on the cited teachings, would not recognize that this technique could be successfully applied to nucleic acids, even if it is effective for proteins and peptides, and thus there is no motivation to incorporate an anion other than the nucleic acid in the proposed composition. Accordingly, there is neither motivation to make this combination, nor a reasonable expectation of success, based on the art of record.

For the above reasons, Applicants submit that the Examiner has failed to set forth a *prima facie* case of obviousness, and accordingly request reconsideration and withdrawal of this rejection.

Claims 40-47 are rejected under 35 U.S.C. 103(a) as being unpatentable over Leong et al in view of Leong et al. or Roy et al. and Beer et al. Applicants respectfully traverse this rejection.

Claim 40 is directed to a method for preparing a gene delivery system by preparing a first solution of a cationic molecule and a second solution of an anionic molecule, adding to either the first solution or the second solution a nucleic acid; and adding to either the first solution or the

second solution a delivery agent, combining the first solution and the second solution to form a third solution comprising the nucleic acid and the delivery agent, and isolating coacervates formed from a portion of said cationic molecule and a portion of said anionic molecule from the third solution, so that the coacervates encapsulate at least a portion of the nucleic acid and the delivery agent.

The teachings of Leong et al., Roy et al., and Beer et al. are described above. Leong et al. (U.S. Patent No. 5,759,582) teach a coacervate microsphere comprising gelatin and chondroitin sulfate incorporating a pharmaceutically active substance, and a method for preparing them by (a) providing a gelatin aqueous solution; (b) providing a chondroitin sulfate aqueous solution; (c) adding a therapeutically effective amount of a pharmaceutically active substance either to the solution in step (a) or to the solution in step (b); (d) mixing the gelatin and chondroitin sulfate solutions to form a coacervate suspension; (e) adding a crosslinking agent to the coacervate suspension to crosslink the coacervates, the coacervates encapsulating the pharmaceutically active substance; and (f) incubating the coacervate suspension to form microspheres and recovering the microspheres.

As noted above, the Examiner has provided no teachings which would suggest 1) that coacervate nanospheres can be formed around viral particles; and 2) that viral particles incorporated in coacervate nanospheres would retain their activity and facilitate intracellular delivery of nucleic acids. Although Beer et al. suggest sustained release of adenovirus from a biodegradable polymer, they point out that they used gentle methods for encapsulation in consideration of mechanical forces on encapsulation (page 63 column 2), obtained spheres >10-20 μm in size, and never specifically suggest the use of coacervates. In contrast, the nanospheres taught by Leong et al. or Roy et al. are orders of magnitude smaller (200-750 nm), possibly too small to encapsulate large viral particles efficiently.

Moreover, Leong et al. espouse the use of non-viral delivery techniques for ease of synthesis, cell-tissue targeting, low immune response, and unrestricted plasmid size, and thus teach ***against*** this combination. Accordingly, there is neither motivation to make this combination nor a reasonable expectation of success based on the art of record. For the above

reasons, Applicants submit that the Examiner has failed to set forth a *prima facie* case of obviousness, and accordingly request reconsideration and withdrawal of this rejection.

CONCLUSION

For the foregoing reasons, Applicants respectfully request reconsideration and withdrawal of the pending rejections. Applicants believe that the claims are now in condition for allowance and early notification to this effect is earnestly solicited. If any questions are raised by the submission of this paper, the Examiner is urged to telephone the undersigned at the number indicated below.

If there are any other fees due in connection with the filing of this Response, please charge the fees to our **Deposit Account No. 06-1448**. If a fee is required for an extension of time under 37 C.F.R. § 1.136 not accounted for above, such an extension is requested and the fee should also be charged to our Deposit Account.

Respectfully submitted,
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Dated: October 4, 2000

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